Programmable Epigenomic mRNA Therapeutics Enable Multi-gene Up or Down Tuning of α-globin via Regulatory Element Targeting: A Precision Approach for β- and α-Thalassemia Treatment

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Platform overview

Therapeutic modulation of the epigenome presents significant opportunities to leverage natural mechanisms to control and resolve gene dysregulation pre-transcriptionally. Omega Therapeutics, a clinical-stage biotechnology company, is designing programmable epigenomic mRNA therapeutics through an innovative platform capable of specifically, controllably and durably modifying epigenetic state to correct aberrant gene expression and treat disease.

Leveraging 3D chromatin architecture, we identify Insulated Genomic Domains (IGDs – loops of chromatin structure containing genes and their regulatory elements), target regulatory elements driving epigenetic gene control within these IGDs, and rationally design Epigenomic Controllers (ECs), mRNA-encoded therapeutic proteins (Fig. 1). Using endogenous modifications, ECs induce changes processed by cellular machinery to tune expression levels of one or more genes.

ECs have been applied to diverse indications, including the investigational mRNA therapeutic OTX-2002 in hepatocellular carcinoma, which is currently under clinical evaluation in a Phase 1/2 trial (NCT05497453). A recent publication (DOI: 10.1038/s41467-024-52202-y) describes its effects on tumor growth in cellular and animal models.

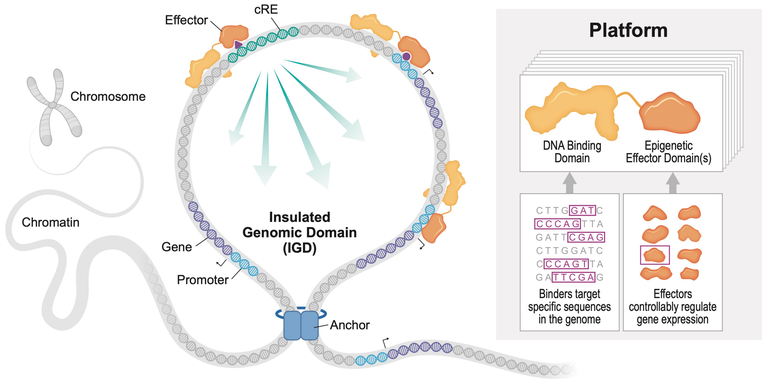

Figure 1. Composition and mechanism of action of epigenomic controllers

β- and α-Thalassemia

β- and α-Thalassemia are rare genetic hemoglobinopathies affecting millions of individuals globally. Worldwide, about 100,000 people with these conditions rely on chronic transfusions, which can have severe complications over time. While hematopoietic stem cell transplantation and gene therapies are a potential option in β-Thalassemia, the therapeutic burden can be high from conditioning and other factors, and results in high rates of infertility. No approved therapy exists for α-Thalassemia.

Given this landscape, there is significant unmet need for treatment options that may reduce transfusion dependence; particularly for therapeutics with significantly lower side effects than current options.

Molecular targeting of β- and α-Thalassemia

β- and α-Thalassemia can result from disequilibrium in expression of the component genes in the hemoglobin tetramer. In the context of β-globin mutations, targeting α-globin offers a direct therapeutic approach, but it is comprised of two genes, HBA1 and HBA2, whose expression is tightly regulated epigenetically.

An effective α-Thalassemia treatment must upregulate both HBA1 and HBA2 to similar levels while under epigenetic regulatory pressure. Conversely, β-Thalassemia treatments that compensate for β-globin mutations may still suffer from a pathogenic imbalance of too much α-globin, leading to anemias. Here a benefit may be realized by inhibiting both HBA1 and HBA2, however the target level of expression should fall within a specific specific range of 25-50%.

This narrow window for therapeutic intervention, across multiple genes, presents challenges for traditional modalities, however ECs present an attractive solution to such gene control.

An Omega approach for multigene control and precise tunability

Gene expression is naturally regulated via elements such as promoters, cis-Regulatory Elements (cREs), and loop anchors. With an ability to target all types of elements, ECs can leverage these different mechanisms to tune expression of one or multiple genes to desirable levels. For example, targeting an individual cRE can induce multigenic or even cell-type-specific effect (Fig. 2).

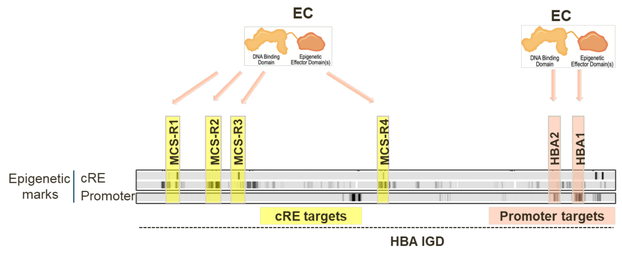

Figure 2: EC targeting of cis regulatory elements (cRE) and promoters, highlighted in yellow and orange, influence the tuning of HBA1 and HBA2 transcriptional expression

In addition, combinatorial targeting of multiple regulatory elements can permit finer tunability in gene transcription control. For example, HBA2 expression can be modulated both up and down by selection of effector and regulatory element target, including additivity observed via simultaneous targeting of cREs and promoters (Fig. 3).

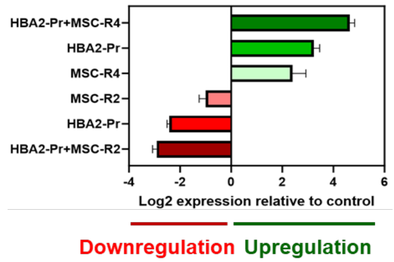

Figure 3: Targeting strategy achieves diverse gene expression control level (red: inhibitory effector, Green: activating effector)

Multi-gene upregulation for α-Thalassemia

Single or multi-gene upregulation can be achieved through choice in cRE targeting. At the HBA1/HBA2 IGD, we identified that targeting MCS-R1 for activation preferentially upregulated HBA1, while targeting MCS-R4 for activation upregulated both HBA1 & HBA2 significantly (Fig. 4). The enrichment of activation histone signatures at the EC target locations validate the intended epigenetic activity of our ECs, and primary mechanism of action (MoA) for gene control.

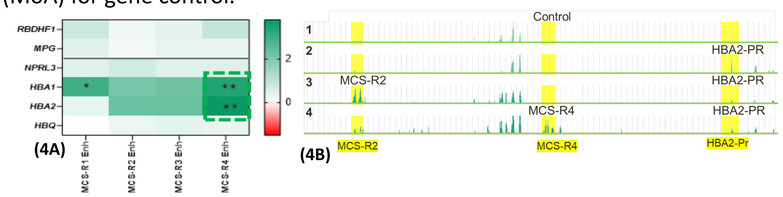

Figure 4: (4A) Color scale indicates level of upregulation (log2 FC) per gene given targeting of 4 different cREs (columns each cRE, rows each gene). **(4B)** Activation signature after EC targeting of HBA2 promoter (2), HBA2 promoter in combination with MCS-R2 cRE (3), or HBA2 promoter in combination with MCS-R4 cRE (4). Enrichment of activation marks at the target loci at 48 hours after treatment with EC.

Multi-gene downregulation for β-Thalassemia

Single or multi-gene downregulation can be achieved through targeting of the same cREs in the HBA1/HBA2 IGD. In specific, targeting MCS-R1 for repression selectively downregulated HBA2, while targeting MCS-R2 for repression downregulated both HBA1 & HBA2 significantly (Fig. 5; published genetic evidence also associates MCS-R2 with α-globin expression). Enrichment of repressive histone signatures at the EC target locations validate the epigenetic activity of our ECs and primary MoA for gene control.

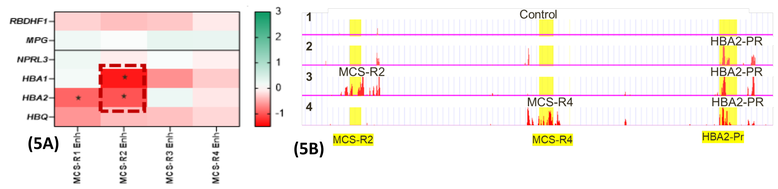

Figure 5: (5A) Color scale indicates level of downregulation (log2 FC) per gene given targeting of 4 different cREs (columns each cRE, rows each gene). **(5B)** Repressive signature after EC targeting of HBA2 promoter (2), HBA2 promoter in combination with MCS-R2 cRE (3), or HBA2 promoter in combination with MCS-R4 cRE (4). Enrichment of repressive marks at the target loci at 48 hours after treatment with EC.

Summary

Our findings suggest a method for directly controlling α-globin levels, at precisely tuned therapeutically relevant levels, through multi-gene epigenomic control. This relies on the differentiated capability to directly target cis-regulatory elements, on their own or in combination. Moreover, the same platform approach can be applied to move these genes up or down to a desired level of expression for potential benefit across related indications.